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Light and Scanning Electron Microscopical Study of Stomatogenesis in the Cyrtophorid Ciliate *Chlamydodon mnemosyne* Ehrenberg, 1837

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Summary. Using Fernández-Galiano silver impregnation technique and scanning electron microscopy of artificially deciliated specimens the divisional morphogenesis of the cyrtophorid ciliate *Chlamydodon mnemosyne* was investigated. Morphogenesis in this ciliate is of the merotelokinetal type with four postoral kineties and two left somatic kineties involved in the formation of the oral ciliature in the future opisthe. Like in other cyrtophorid ciliates (*Trithigmostoma*, *Chilodonella* and *Brooklynella*) the stomatogenic kinetofragments perform a complex morphogenetic migration to yield the inverted preoral kinety and the inner and outer circumoral kineties. The differentiation of an oral rosette made of 9-11 large and two smaller nematodesmal rods covered by a prominent collar of cytostomal corrugations is described. Oral reorganisation in the proter, involving dedifferentiation of the entire parental cytostome and the *de novo* formation of a new cytostome in front of the old one is best seen in deciliated dividers. Replication of kinetosomes in particular the transformation of somatic monokinetids into oral dikinetids as judged from light microscopy seems similar to the events in *Trithigmostoma*. Details observed in *Chlamydodon* are discussed in comparison with other cyrtophorid ciliates.

Key words: artificially deciliated ciliates, *Chlamydodon mnemosyne*, Cyrtophorida, morphogenesis, oral reorganisation, scanning electron microscopy.

INTRODUCTION

Chlamydodon mnemosyne Ehrenberg, 1837 is an euryhaline cyrtophorid ciliate which can easily be cultivated in 50 % sea-water with filamentous cyanobacteria as food (Kurth and Bardele 2001). Pulse-fed mass cultures provided enough cells in various stages of binary division to study divisional morphogenesis. Stomatogenesis in *Chlamydodon* as in all other Cyrtophorida belongs to the merotelokinetal type. As described in detail both at light and electron microscopical level for *Chilodonella cyprini* (Hofmann 1987) and *Trithigmostoma steini* (Hofmann and Bardele 1987) only a few somatic kineties are involved in the formation of the new mouth of the posterior daughter cell, the opisthe. Kinetofragments of these stomatogenic kineties which consist of monokinetids separate from the rest of the ventral somatic kineties and transform into dikinetids. Subsequently these kinetofragments assemble into three entities which perform a complex morphogenetic migration around the position of the future oral area, resulting in three inverted perioral kineties known as the inner and

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outer circumoral kinety and the preoral kinety. During this morphogenetic movement parts of these kineties separate to nucleate the new nematodesmal rods of the cytopharyngeal basket and the microtubular cytopharyngeal lamellae which form the inner tube of the basket. Earlier light microscopical studies on stomatogenesis e.g. by Lom and Corliss on *Brooklynella hostilis*, an ectoparasite on gills of marine fishes (Lom and Corliss 1971), and several other dysteriid ciliates by Deroux (1994) support a fairly uniform stomatogenesis in the opisthe of cyrtophorid ciliates.

Contrary to the well-understood processes in the opisthe almost no information is available about reorganisation of the oral apparatus in the proter. It is the aim of this study to add new data here. As a prerequisite for thin-section studies currently in progress we give a description of the light microscopical aspects of stomatogenesis in *Chlamydodon mnemosyne* supplemented with SEM observations on artificially deciliated specimens, which were of great help to study the asynchronous events in the two daughter cells, the proter and the opisthe.

MATERIALS AND METHODS

The origin of cultures of *Chlamydodon mnemosyne* and the methods to grow this ciliate have been given in the preceding paper (Kurth and Bardele 2001). In order to increase the number of cells in division starved cultures were pulse-fed, but no long-term synchronisation was reached. For light microscopy bulk samples were stained with pyridinated silver carbonate according to Fernández-Galiano (1976) with the following modification. Cells were first fixed with 1 % formaldehyde and then stored in 0.6 % formaldehyde for several days in the refrigerator before the standard procedure was continued. For SEM cells were fixed in Párducz solution (Párducz 1967) and processed as described in Kurth and Bardele (2001). Prior to fixation cells were artificially deciliated by mechanical agitation for 3 min with a Pasteur pipette in a solution of 4 % ethanol in a 1:1 mixture of Eau Volvic (French mineral water from the Auvergne) and natural seawater. The deciliated cells allowed us to describe in more detail than in silver-stained preparations the asynchronous morphogenetic events in both the proter and the opisthe.

RESULTS

Prior to the description of morphogenesis we give a short summary of the general morphology of *Chlamydodon mnemosyne*. The cell measures about 70 x 45 µm. The slightly curved dorsal face is completely free of cilia. At the borderline between the ventral and the dorsal face of the cell there is the genus-specific "striped band" of unknown function, colloquially called "railroad track" (MacDougall 1928). The ventral surface of the cell is flat and completely ciliated except for the oral area.

The somatic ciliature on the ventral surface consists of a right and a left field of somatic kineties with four postoral kineties in between. The right somatic field consists of 12-15 kineties, their anterior ends bend over to the left anterior corner of the ventral surface and abut to the anterior ends of the left somatic kineties, 9-11 in number. The somatic kineties are made of monokinetids displaying the typical cyrtophorid kinetid pattern (Lynn and Small 1981). The most characteristic feature of the somatic kinetids are the sheets of subkinetal microtubules which overlap in an anterior direction (for details see Kurth and Bardele 2001).

The slit-shaped cytostome is located in the anterior third of the ventral surface. The cytostome is surrounded by an oval collar of solid corrugations. In front of the cytostome are three more or less straight inverted perioral kineties, the inner and outer circumoral kinety and the preoral kinety, all three made of dikinetids with only the anterior kinetosome ciliated. The cytopharyngeal basket consists of 9-12 nematodesmata and an inner ring of cytopharyngeal microtubular lamellae. The nematodesmata are capped each by a so-called dens (or capitulum) which contains a barren kinetosome.

We found it convenient to divide morphogenesis of *Chlamydodon* into nine stages (Fig. 1). This somewhat unusual way to present the summary first helps to orientate the readers and facilitates the description of details. Note that Fig. 1 shows only the middle part of the ventral face, which is the area where the important changes take place during morphogenesis. The course of stomatogenesis is not synchronous in both the future anterior and posterior daughter cell, the proter and the opisthe. In the description of the morphogenetic stages we first mention the events which take place in the opisthe. Changes in the opisthe are more complex than the changes in the proter which are restricted to the reorganisation of the parental cytopharyngeal complex.

The formation of the oral structures of the opisthe is entirely from somatic components. No constituents of the parental oral structures are involved. A special role is played by the four postoral kineties, labelled 1 through 4 in Fig. 1, also involved are single kinetosomes of the somatic kineties to the cell's left, labelled 5 and 6. Since

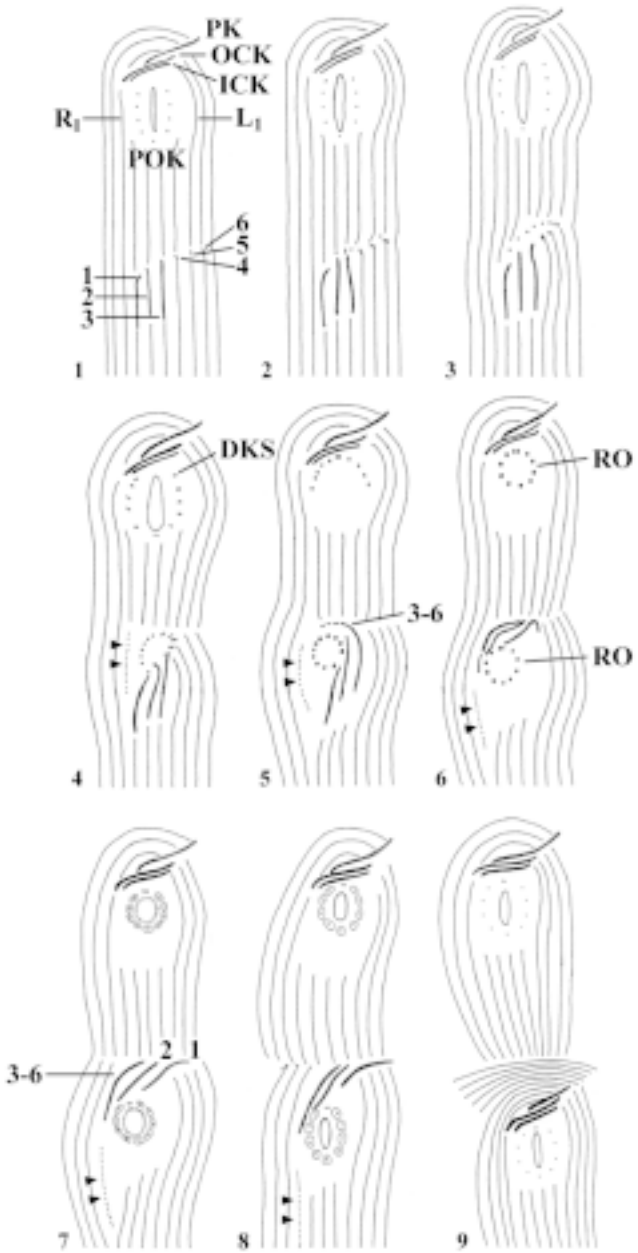
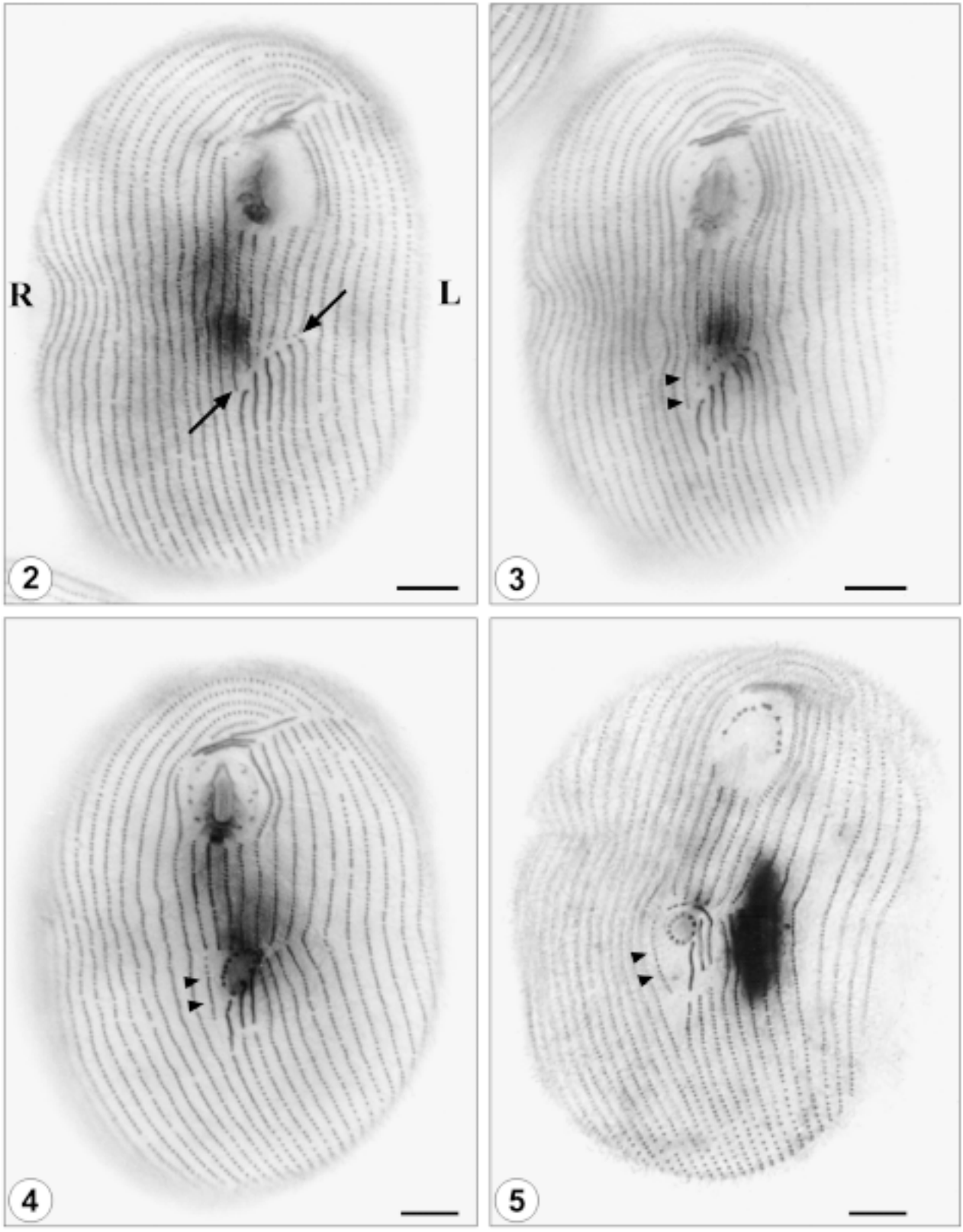


Fig. 1. Diagram of the morphogenesis in *Chlamydodon mnemosyne*. Nine stages labelled 1 through 9 are recognised. Note that only the middle part of the ventral surface is shown. In subfigure 1 the perioral ciliature consisting of the preoral kinety (PK), the outer circumoral kinety (OCK) and the inner circumoral kinety (ICK) are shown by a thicker line, since these three kineties are made of dikinetids. Also the first three segments (labelled 1-3) of the four postoral kineties are shown in thicker lines since in these segments somatic monokinetids transform into dikinetids. These segments will perform a complex morphogenetic migration and become the perioral ciliature of the opisthe (see text for details). The three kineties to the left, labelled 4-6 (or K4 - K6 in text), are also involved. Single kinetosomes or pairs of kinetosomes which lie in front of the stomatogenic fragments become more distinct. In the subsequent stages of stomatogenesis these kinetosomes form the so-called rosette which nucleates the nematodesmata of the cytopharyngeal basket of the opisthe. The cross-sectional profiles of the nematodesmata are shown as small circles round the kinetosomes of the rosette (subfigures 7-8). Actually, the rosette kinetosomes are localised in fibrous dents sitting on top of the nematodesmata. Reorganisation of the oral structures in the proter is characterised by the complete disassembly of the parental nematodesmata (not shown) by significant changes in the array of the rosette kinetosomes. Note the intermediate opening of the circle into a crescent line (subfigure 5), its subsequent closure, and re-assembly of the nematodesmata in the proter. In *Stage 1* to *3* in the proter, and in *Stage 9*, both in proter and opisthe, only few details are seen of the cytostome-cytopharynx complex due to the fact that the corrugations of the collar obscure the structures lying underneath. Further abbreviations: DKS, double kinetosome; L1, first left kinety; POK, postoral kineties; R1, first right kinety; RO, rosette. Starting from subfigure 4 through 8 a middle segment of the first postoral kinety is shown as a dotted line and labelled with two arrowheads. This "new" kinety in the opisthe compensates for the fact that the opisthe does not get a part of the leftmost parental somatic kinety (not shown) because it is too short to reach the opisthe. In some preparations pairs of kinetosomes can bee seen in the cytopharyngeal region of the proter as depicted in subfigure 4

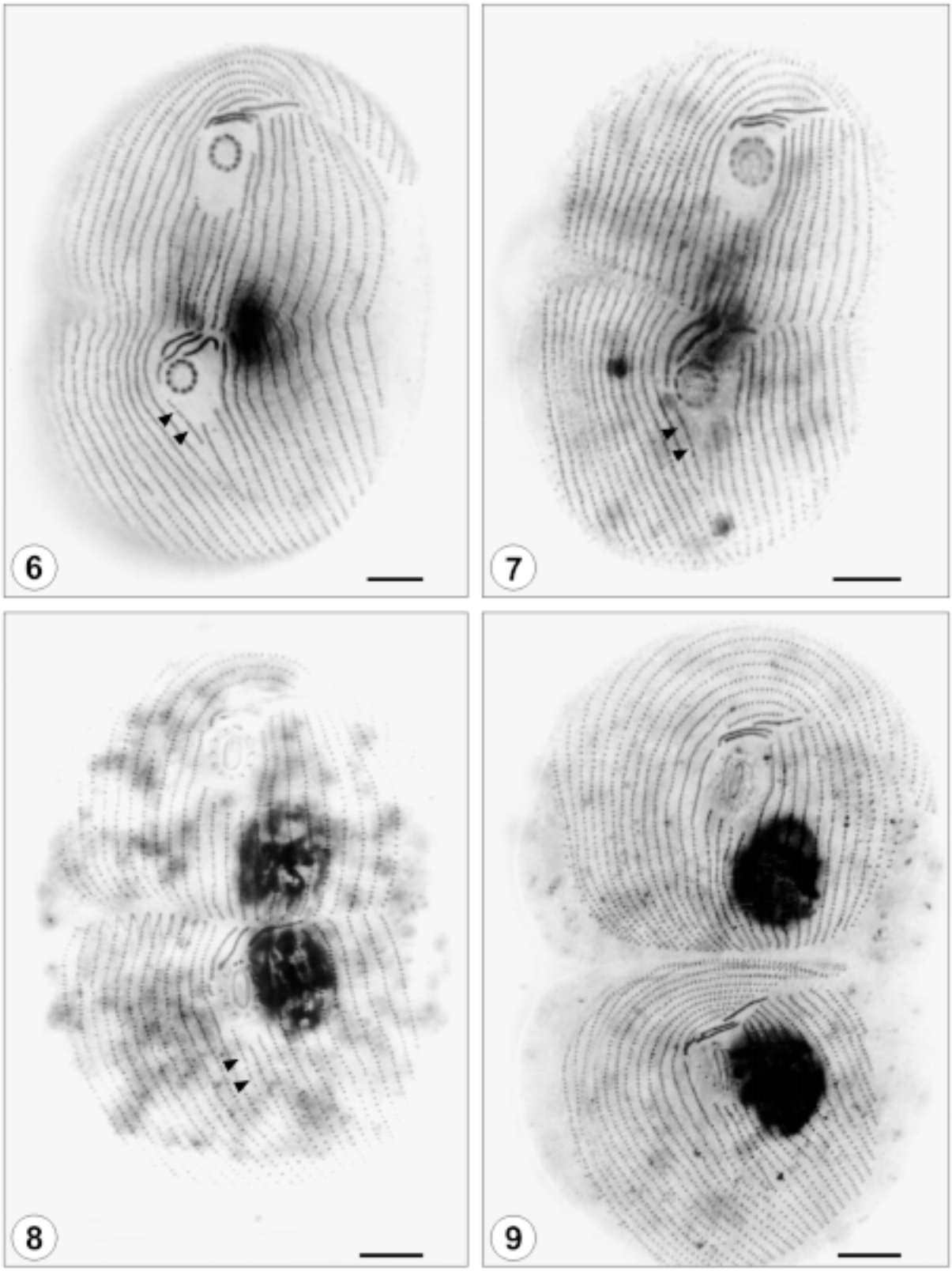
both at light microscopical and at SEM level no visible changes are seen in the proter during *Stage 1-2* the first events concern the opisthe only.

Stage 1. As the first sign of the beginning of stomatogenesis in *Chlamydodon* an oblique interruption of the straight course in the postoral kineties is seen immediately behind the middle of the ventral surface of the cell. This stage is not shown in a silver-stained specimen, it corresponds to the cell outlined in the upper left corner of Fig. 1. The three kinetofragments labelled 1-3 and the three pairs of kinetosomes shown anterior to the fragments labelled 4-6 will become the three perioral kineties of the opisthe. Note that in the following eight micrographs of silver-stained cells the number of the figure is identical with the morphogenetic stage it represents, thus e.g. Fig. 2 shows *Stage 2*, and so on.

Stage 2. The oblique interruption anterior to the stomatogenic kineto-fragments (K1 through K6) is seen more clearly (Fig. 2). Since they appear thicker than the other kineties they probably have replicated their kinetosomes. During the further course of stomatogenesis the three thicker parts, called hitherto stomatogenic kinetofragments, will separate from their anterior and posterior endings. Stomatogenic kinetofragment number 1 is the first to show its anterior end bending to the left (Fig. 2). Moreover, the very anterior ends of K4 to K6 appear more dense than the rest of the kineties. At times, pairs of kinetosomes were seen in this area, as drawn in Fig. 1 (subfigures 1, 2). Finally, several argentophilic dots, probably representing single kinetosomes, are seen at the posterior ends of the anterior fragments of the postoral kineties in an area close to the future



Figs. 2-9. *Stage 2* through *Stage 9* of morphogenesis of binary fission in *Chlamydodon mnemosyne* stained with pyridinated silver carbonate according to Fernández-Galiano; arrows in Figure 2 indicate the oblique interruption in front of the stomatogenic kinetofragments; arrowheads indicate the ”new” kinety forming the prospective first postoral kinety of the proter. For details see text. Scale bars - 10 µm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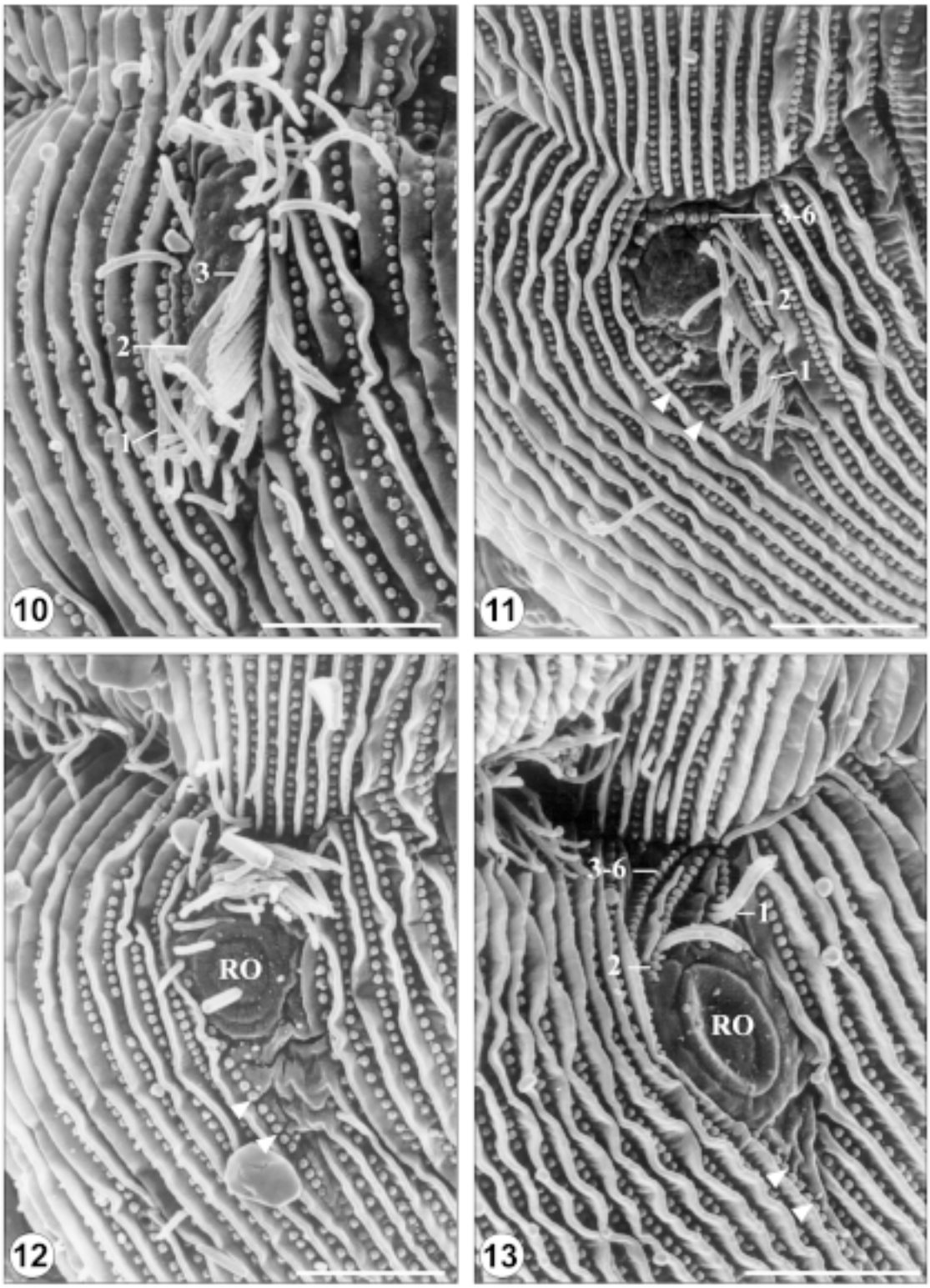
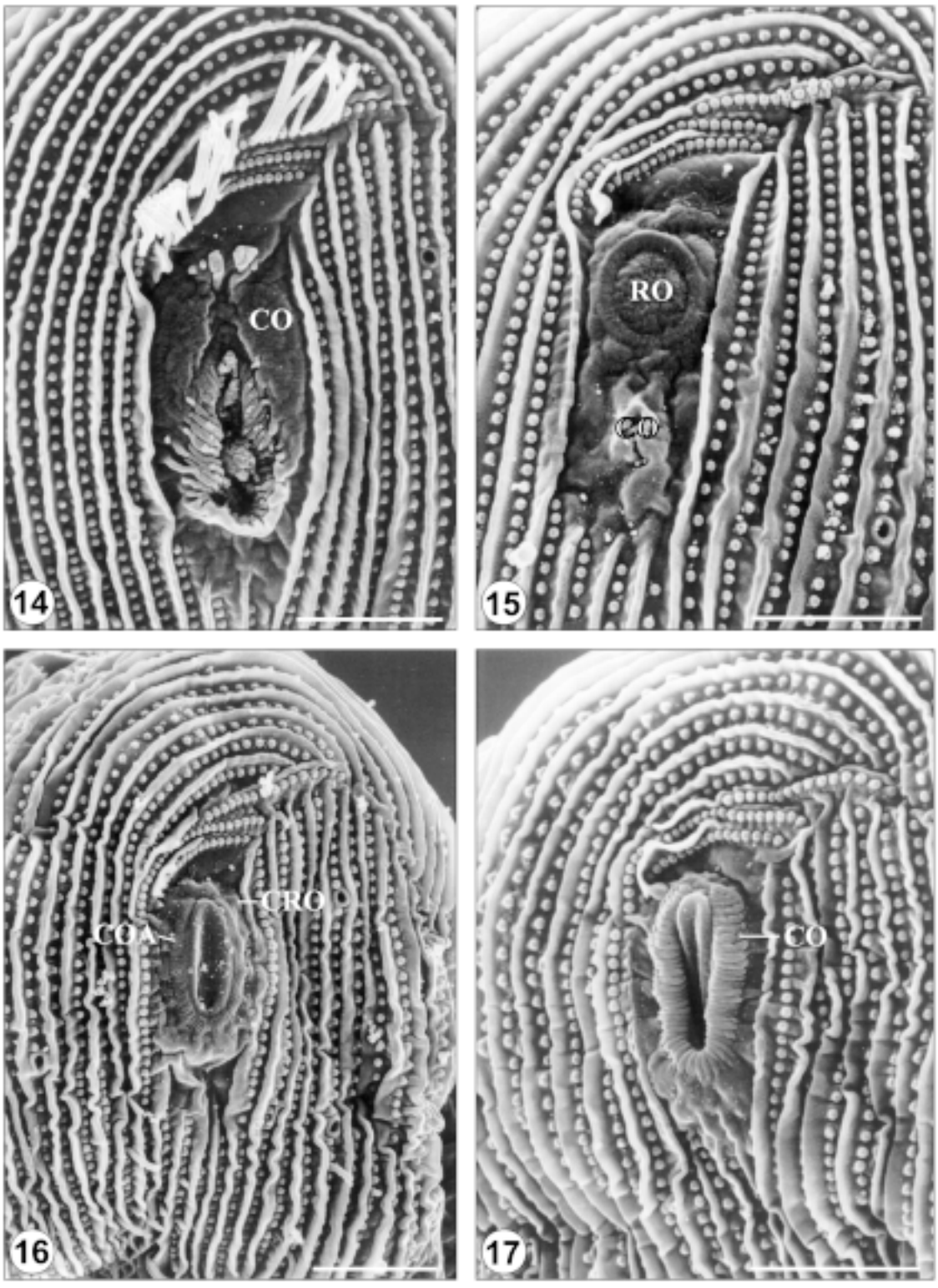


Figs. 10-13. SEM view of artificially deciliated cells showing the morphogenetic events in the opisthe starting with *Stage 4* (**10**) and ending with *Stage 7* (**13**). For unknown reasons the perioral cilia sometimes withstand the deciliation procedure. Thus in (**10**) the three stomatogenic kinetofragments 1 to 3 show cilia. In (**11**) the compound of kinetofragment 3-6, later becoming the preoral kinety, has moved around the future rosette. Note that the perioral kineties show a single row of ciliary stubs more closely spaced than the somatic cilia. The second barren basal body of each perioral dikinetid cannot be shown with this technique. The ”new” first postoral kinety of the opisthe is labelled with two white arrowheads in (**11-13**). In *Stage 6* (**12**) the oral rosette (RO) is labelled. This latter structure is seen more clearly in (**13**), where it shows an inner slightly higher rim and a flatter outer rim, from which the corrugations of the collar will develop. Scale bars - 5 µm



Figs. 14-17. SEM view of the reorganisation of the cytostomal area in the proter after artificial deciliation. **14** - invagination and resorption of the parental collar (CO); **15** - a new rosette (RO) has formed anterior to the old cytostomal area; **16** - differentiation of the corrugations or the collar anlage (COA) and cytopharyngeal rods (CRO); **17** - the corrugations of the collar (CO) have almost reached their final shape. Scale bars - 5 µm

posterior end of the proter. All these dots (or kinetosomes) arrange themselves in a slightly crescent line in the following stage.

Stage 3. The gap between this line of single kinetosomes and the anterior end of the stomatogenic kinetofragments widens. Another important feature is the elongation of the first anterior postoral kinetofragment by proliferation of its posterior kinetosomes in a backward direction (two arrowheads in Fig. 3). It is in *Stage 3* that for the first time changes in the proter become obvious. The oral slit, till now very small, widens gradually. The oval array of kinetosomes, which are located in the dentes heading the nematodesmata, assume a U-shaped array (Fig. 3). The central area of the cytostome region looks empty, probably due to the detaching of the cytopharyngeal basket from the prior cytostome.

Stage 4. In the future oral area of the opisthe, single kinetosomes and one or two pairs of kinetosomes begin to arrange themselves in a circular array (Fig. 1, subfigure 4 and Fig. 4). Other pairs of kinetosomes or derivatives seemingly originating from kinetofragment 4 to 6 are now positioned anterior to the stomatogenic kinetofragment 3. The anterior ends of all three major stomatogenic kinetofragments are bowed to the left side of the cell and seem to start their morphogenetic migration around the position of the future oral area of the opisthe (see also Fig. 10). The rightmost postoral kinety in the future proter has grown in length. The nematodesmal kinetosomes are duplicated resulting in pairs of kinetosomes (Fig. 1, subfigure 4) consisting of an old and a new kinetosome. (Note that the scanning electron microscopical aspects of *Stage 4* through *Stage 7* of the opisthe are illustrated in Figs. 10-13.)

Stage 5. This stage is an intermediate stage of the morphogenetic movement of the stomatogenic kinetofragments. Kinetofragment 3 is the quickest in its movement around the now circular ”rosette”, the nucleation site of the cytopharyngeal rods (Fig. 5). The term rosette was introduced by Lom and Corliss in their study of *Brooklynella* (Lom and Corliss 1971), later such an array of kinetosomes was also observed in *Chlamydonella*, *Lynchella* and *Chilodonella* (Deroux 1976, 1994). At the anterior end of kinetofragment 3 short streaks of argentophilic elements are seen. These are presumably the derivatives of K4 through K6 first seen in *Stage 2* and probably represent replicated kinetosomes. This interpretation is influenced by our knowledge of the corresponding events in *Trithigmostoma* and *Chilodonella* (Hofmann 1987, Hofmann and Bardele 1987). It is a derivative of K6 which leads the elephant dance to the right around the oral rosette, followed by elements of K5 and K4, while kinetofragment 3 forms the tail. Altogether K3, K4, K5 and K6 (labelled 3-6 in Fig. 1, subfigure 5 and Fig. 11) will form the future outer circumoral kinety of the opisthe. Note that in *Stage 4* and *Stage 5* the somatic kineties which form the inner part (close to the mouth) of the somatic ciliature have assumed a slightly bowed configuration in preparation of their course in the mature opisthe (Figs. 4, 5).

In the proter the oral slit has become invisible in silver-stained specimen but more important the kinetosomes formerly associated with the dens, and once sitting on top of the nematodesmata have assumed a semicircular linear array with one or two pairs of kinetosomes in the middle of the arc. Compared to *Stage 4* the array of kinetosomes in the proter of *Stage 5* now shows an inverted U-shape. From observation of living cells we know that in *Stage 5* the cytopharyngeal basket has separated from the cytostome, and sunken into the cytoplasm where it starts to disassemble. It is probably through the separation of the basket from the dentes that the kinetosomes can change their arrangement. But quite unexpectedly, the circle of kinetosomes obviously opened at its posterior end and not at the anterior end where two small cytopharyngeal rods were observed in thin sections (Kurth and Bardele 2001). Finally, at least in *Stage 5* the beginning of the fission furrow cuts in from the right side of the cell.

Stage 6. In this stage the oral rosette is complete. In most cases it consists of 11 kinetosomes (Fig. 6), two of them difficult to see in silver-stained preparations, but known from TEM (see inset in Fig. 19 in Kurth and Bardele 2001), lie close to each other at the anterior end of the rosette. Though circular in outline (see Fig. 12) a bilateral structure has formed, as an announcement of the bilateral symmetry of the oral collar seen in the mature cell. Very complex rearrangements of the future circumoral kineties take place in *Stage 6* and the following stages, which for lack of space cannot be documented in every detail. The migration of the future perioral kineties, when seen from the ventral side, is in a counter-clockwise fashion. The anterior ends of all three perioral kineties point to the right side of the cell and during further migration their anterior ends point to the posterior end of the cell while the posterior ends of these same kineties point in an anterior direction, thus resulting in an inverted position of the perioral kineties. In particular K1 which later will become the preoral kinety, has a distinct position in *Stage 6*, its anterior end lies anterior to the apex of the rosette while its posterior

end forms a hook (see Fig. 1, subfigure 6 and Fig. 6). Otherwise, the cell shown in Fig. 6 is somewhat untypical, the opisthe shows only two (instead of three) postoral kineties and the third being added to the right of the former.

As far as the proter is concerned the open arch of kinetosomes from *Stage 5* has now closed again to re-establish the circular array of the rosette. The changes which occur on the surface of the oral area of the proter are seen best in deciliated specimens. The oral collar of the parental mouth is invaginated beginning with the anterior part as shown in Fig. 14. Moreover, Fig. 6 (upper right) shows the short length of the leftmost somatic kinety, which consists of eight kinetids only in this particular micrograph. They all will end up in the proter which is a typical behaviour of the leftmost somatic kinety in other cyrtophorid ciliates, likewise (Deroux 1994). The solution of this problem, loss of one somatic kinety in the opisthe, comes from the observation of the behaviour of the "new" kinety which slides down past the right of the future oral area of the opisthe (arrowheads in Fig. 1, subfigures 4-8 and Fig. 11) and enters the V-shaped empty space seen on the right side of the three postoral kineties in Figs. 5, 6, 12.

Stage 7. The hook-like posterior end of K1 has straightened. Its posterior end has moved toward the left part of the fission furrow, into a zone which later becomes the anterior suture. The anterior end of K1 still lies posterior left to K2. A major characteristic of *Stage 7* is the threading of the new first postoral kinety (arrowheads in Fig. 1, subfigure 7, and Fig. 13) between R_1 and the now second postoral kinety of the opisthe.

The majority of rosette kinetosomes in both the proter and the opisthe are now surrounded by a broader halo of dense material, which may indicate the beginning assembly of the nematodesmal rods. This does not hold for the two anterior kinetosomes of the rosettes of both daughter cells, thus resulting in baskets with nine massive rods at light microscopical level. It is noteworthy that thin-sections have revealed two very thin nematodesmata associated with the anterior kinetosomes (not shown). In *Stage 7* all somatic kineties of the former parental cell show a distinct interruption at the level of the future fission zone. The posterior end of the anterior fragments as well as the anterior end of the posterior fragments seem to proliferate their kinetosomes. The oral area of the proter in SEM micrographs now shows the new rosette anteriad of the former mouth region of the parental cell (Fig. 15).

Stage 8. The circular rosettes both in the proter and the opisthe have changed into oval structures. The diameter of the nematodesmata in both cells has enlarged compared to *Stage 7* (Fig. 1, subfigure 7 and 8). In the opisthe K1, the future preoral kinety of the opisthe has further moved to the left side of the cell, but its anterior end is still posteriad the posterior end of K2. The two anterior dots of the oral rosette in the opisthe are particularly dense in this stage (Fig. 8). Thin sectioning has to show whether kinetosome replication occurs at this site. Deciliated specimens of *Stage 8* cells show that in the proter the formation of the new corrugations of the oral collar has begun (Fig. 16).

Stage 9. The new preoral kinety of the opisthe has reached its definitive position in front of the outer circumoral kinety (the former K3-6) which means that K1 has moved with its anterior end ahead to the right of the cell thus moving around the posterior ends of the two other perioral kineties. The anterior part of all right somatic kineties has bowed over to the left side of the cell, abutting the preoral kinety which lies in the anterior suture (Fig. 9). The distal ends of the nematodesmal rods have become undetectable at light microscopical level in both daughter cells probably through differentiation of the corrugations of the oral collar as indicated in Fig. 17. This same figure shows that finally the central part of the former ring-shaped but later oval covering of the cytostome becomes overgrown by the oral collar.

DISCUSSION

Technical remarks

Silver impregnation techniques have a long tradition in the study of morphogenetic events in ciliates. Not all available procedures are equally suited for any particular group. For cyrtophorid ciliates the Fernández-Galiano technique gives a clearer picture of the kinetosomes compared to the Chatton-Lwoff technique (Frankel and Heckmann 1968) while the Protargol technique (Tuffrau 1967) (at least in our hands) gave no satisfying results. We got the impression that the Chatton-Lwoff technique does not stain barren basal bodies as distinctively as ciliated ones. But other than that there is as yet no rational explanation why in a certain group a particular technique is superior to another one.

The deciliation technique combined with traditional scanning electron microscopy are powerful tools to

study divisional morphogenesis. The explanation is straight forward. Ciliates with a distinct layer of epiplasm are particularly well suited for the deciliation technique with 3-4 % ethanol. Cilia break off just above the kinetosome and precisely indicate the former position of a cilium. Barren kinetosomes cannot be demonstrated with this technique as long as they are covered by epiplasm. The dense spacing of the kinetosomes in the perioral kineties compared to the somatic kineties (see e.g. Fig. 16) gives a very realistic view. In addition, we have at times observed a differential persistence of cilia undergoing resorption and re-growth, an interesting process of renewal of oral cilia, to be dealt with later. Likewise, for the illustration of cortical ridges between the kineties as well as the mapping of the openings of the contractile vacuoles the deciliation technique is of great value. This holds also for the visualisation of the dynamic processes which occur both during stomatogenesis and reorganisation of the structures associated with the cytostome proper (Figs. 10-17). Finally, not yet illustrated in this paper, it is quite obvious that certain morphogenetic changes seen in thin sections are much easier to understand if a clear three-dimensional view is at hand.

Divisional morphogenesis regularly comprises two separate events, somatogenesis (replication of the somatic ciliature) and stomatogenesis, the formation of new oral structures for the opisthe. Though in a few cases the old oral structures of the parental cell seemingly without any major alterations, remain with the proter, in an increasing number of ciliates complex reorganisation of the parental oral structures is recognised when studied at greater depth. These processes are more difficult to observe since they occur within an already differentiated structure of the proter. Some entities develop while others, close by, gradually vanish following a timetable difficult to unravel. Static pictures seen in silver-stained preparations, in SEM and likewise in thin sections need to be arranged in a correct sequence. For this procedure to be done it needs special landmarks and key events which can be recognised reliably.

The type of stomatogenesis which is realised in *Chlamydodon* was formerly called "somatic-meridional" since only kinetosomes of somatic kineties are involved (Corliss 1968). Now it is called "merotelokinetal" (Bardele 1989, Foissner 1996) since not all but only a certain number of kinetofragments, which separate from the parental kineties, transform into the oral ciliature of the opisthe. Dedifferentiation and subsequent re-differentiation of the oral structures in the proter are variable in degree.

Among the cyrtophorid ciliates to which *Chlamydodon* belongs we have the most detailed information about divisional morphogenesis on *Chilodonella* studied most carefully by Hofmann (1987). Since the morphogenesis of *Chilodonella* has become a kind of model system for cyrtophorid ciliates important highlights of this study are mentioned again since they are the basis for further discussion: (i) the transformation of somatic kinetofragments originally made of monokinetids into dikinetids, (ii) the documentation of complex morphogenetic migration of these stomatogenic kinetofragments to the area of the future formation of the oral apparatus while at the same time these arrange themselves into the three inverted perioral kineties, (iii) the differentiation of the nematodesmal rods from some specialised kinetosomes and finally (iv) the assembly of the inner ring of cytopharyngeal lamellae from postciliary microtubules originating from somatic kinetids. Thus *Chilodonella* became one of the best understood model systems of ciliate stomatogenesis as far as the new formation of the cytostome in the opisthe is concerned. However there is little information on the reorganisation in the proter. It is this topic from which our interest in *Chlamydodon* came when we had performed the first investigations on deciliated cells.

Initial studies on the morphogenesis of *C. mnemosyne* performed by Fauré-Fremiet (1950) and Kaneda (1960) led to results now prone to be corrected. Both authors describe an anarchic field of irregularly positioned kinetosomes in the area of the future opisthe's cytostome. In our material of *C. mnemosyne* we have seen no such anarchic field. Instead we have always observed a highly ordered migration of the morphogenetic kinetofragments as described for *Trithigmostoma*, *Chilodonella* (Hofmann 1987, Hofmann and Bardele 1987) or *Brooklynella* (Lom and Corliss 1971). At best, the few anterior kinetosomes of K4 through K6 when they align into the tip of the future preoral kinety can give the illusion of a small anarchic field. One speculation could be that during certain steps of the Chatton-Lwoff procedure used by Fauré-Fremiet (1950) desmose-like connections between kinetosomes become weakened to yield a more disordered array of kinetosomes. On the other hand we know from preliminary thin section studies that there is a large number of osmophilic granules of an appropriate size in the oral area of proter and opisthe which are less numerous in non-diving cells. These granules could also obscure the ordered array of

kinetosomes in living or Chatton-Lwoff processed specimens.

The denser appearance of K1 through K3 in Figs. 2-4 is probably due to kinetosome duplication in these stomatogenic kineties. This interpretation is based on thin section studies currently in progress and in accordance with observations made in *Trithigmostoma* and *Chilodonella* where ultrathin sectioning has shown that kinetosome proliferation is accomplished by the "transition" of monokinetids into dikinetids (Hofmann 1987) in the same space. This means that the morphogenetic kineties do not elongate but their doubled kinetosomes are spaced more densely and such display a more distinct appearance.

The migration of the morphogenetic kinetofragments in *Chlamydodon* is similar to those in *Chilodonella*, *Trithigmostoma* and *Brooklynella*. The migration behaviour of K1, however, is more closely fitting to that of K1 in *Brooklynella*, where it reaches its final position relative to the other kinetofragments rather late in development. In *Chilodonella* and *Trithigmostoma* the migration of K1 is retarded and it moves early around the other fragments reaching its final position relative to the other kinetofragments at middivisional stages.

In the proter, Fauré-Fremiet (1950) observed the U-shaped opening of the parental oval array of cytopharyngeal rods. This seems to correspond to our *Stages 2* and *3*; but no inverted-U-shape array (*Stage 5*) has been described in the proter by other investigators. In addition, there are some uncertainties about the reorganisation in the preoral and circumoral kineties in the proter. In *Trithigmostoma* "oral" cilia become resorbed and grow out again (Hofmann and Bardele 1987). The corresponding kinetosomes stay in place. As already indicated such "reorganising" cilia seemingly withstand the deciliation procedure more often than non-reorganising cilia. A more subtle elaboration of "differential deciliation" technique with various ethanol concentrations and varied periods of mechanical agitation might be useful to document the various stages in the life history of a cilium.

Contrary to the more gradual reorganisation of the oral ciliature in *Trithigmostoma* and *Chlamydodon*, in *Brooklynella* a seemingly sudden rebuilding of the oral ciliature seems to take place as judged from light microscopical observations (Lom and Corliss 1971). New kinetosomes for the oral cilia of the proter are said to originate from the anterior tips of the middle postoral somatic kineties. We have not been able to decide whether a similar process occurs in *Chlamydodon*. *Stage 4* to *Stage 5* is the most important period for this event. We have shown in Fig. 1, subfigure 4 and 5 the sudden change of a U-shaped array into an inverted-U-shaped array of kinetosomes, which could be either pairs of kinetosomes or single ones becoming covered by the material of the future dentes and thus appearing more voluminous than ordinary kinetosomes. This remains a problem which ought to be clarified by thin sectioning.

Other types of oral replacement, e.g. the reorganisation of the paroral (or undulating) membrane in *Tetrahymena* (Nelsen 1981) likewise display subtle changes, and took quite a time before they were realised as regular phenomena in divisional morphogenesis. Reorganisation of parental adoral organelles is the most difficult process to be documented. From unpublished work on the colpodid ciliate *Platyophrya* we know that single cilia within an adoral organelle become resorbed and grow out again, a process which was only seen in Cryo-SEM studies.

Another very dramatic event is the detachment of the cytopharyngeal basket in the proter (see Kaneda 1960). Its dissolution in the cytoplasm as well as the formation of the new basket could not be studied with silver impregnation or with SEM, these processes must be clarified by thin section studies. Unfortunately, in light microscopical studies, both the beginning of the detachment of the basket and likewise the first signs of its new formation are hidden by the massive corrugations of the oral collar (Kaneda 1960).

With respect to the timetable and contrary to the drawing by Fauré-Fremiet (1950) there is no synchronous development in the differentiation of oral structures of the opisthe and the proter. As seen most clearly in our Fig. 5 the opisthe is ahead of the proter. We know of no published explanation for the asynchronous development in proter and opisthe which ought to be a very general problem encountered also in any form of asymmetric cell division.

Morphogenetic events in the somatic cortex are less spectacular. The migrating fragment of the first postoral kinety (dotted line in Fig. 1, subfigures 4-8, labelled with two arrowheads) is of special significance for the completion of the full number of the somatic kineties since the leftmost somatic kinety due to its short length has ended up completely in the proter. This type of kinety compensation has also been described for *Chilodonella*, *Trithigmostoma* and *Brooklynella* and is of considerable significance for lateral turnover of somatic kineties, at least for the opisthe. Though this mode of cortical shift is regarded by Deroux (1994) as a synapomorphic

character of the Cyrtophorida, lateral turnover of somatic kineties seems to be more widely distributed among ciliates. One of the most impressive examples of lateral turnover is the cortical slippage of artificially inverted kineties in *Paramecium tetraurelia* (Beisson and Sonneborn 1965). Lateral turnover is a general morphogenetic feature leading to a complete renewal of the cortex in 20 - 30 generations in normal cells of *P. tetraurelia* (Iftode and Adoutte 1991). Moreover, a longitudinal turnover of the somatic cortex of ciliates is implicated in the "clonal cylinder" model by Frankel (1989).

Neither at light microscopical level nor with the SEM we have seen any indication of the growth of the cross-striped band. In mid-divisional stages there is a more pronounced indentation on the right side of the cell than on the left side at the height of the future fission furrow. Further thin section studies have to show whether this indentation which sometimes looks like a small cavity is involved in the necessary increase of the C-shaped elements of the so-called railroad-track.

Phylogenetic remarks

In continuation of our aims to reconstruct ciliate phylogeny through comparative studies on ciliate morphogenesis we are constantly puzzled by the quite unique type of oral ciliature in cyrtophorid ciliates. At first glance there is a certain resemblance of the oral apparatus of the Cyrtophorida with the Nassulida. But this resemblance only holds for the basket. Nassulids show a paroral and an adoral ciliature, found in all ciliates with the exception of the Phyllopharyngea and the Litostomatea. While it seems most likely that the Litostomatea have lost their primary oral ciliature and substituted it through "oralisation" of somatic ciliature (Foissner and Foissner 1988), in the Phyllopharyngea a more cryptic way of "deuterostomisation" might have happened. The current oral ciliature, the preoral kinety and the circumoral kineties, display substantial differences to a paroral or an adoral ciliature. Though derived from the somatic ciliature they must have had some other kind of secondary origin together with the cytostome proper. We envision the following scenario: primitive phyllopharyngids, no longer existent, had lost their primary oral ciliature and their primary cytostome and developed suctorial tentacles, thus Suctoria being a very basal branch of the Phyllopharyngea and not on top as usually argued. Some suctorians form microtubular lamellae which are major components of their tentacles in close neighbourhood to basal bodies (Hitchen and Butler 1973). These microtubular lamellae we regard as homologous to the cytopharyngeal lamellae in cyrtophorids which originate from postciliary microtubules of somatic kinetosomes (Bardele 1987, Hofmann 1987). As oralised kinetosomes in litostomes or kinetosomes of ophryokineties (Didier 1971) can form nematodesmata, somatic kinetosomes form cytopharyngeal rods in nassulids (Tucker 1970, Peck 1974, Eisler 1989) and by convergence in cyrtophorids (Hofmann 1987). The cytostome in cyrtophorids (and consequently also in chonotrichids) is a secondary one and the oral kineties in both are not homologous to the paroral or adoral ciliature of other ciliates. The main purpose of their kinetosomes at least during a certain period of morphogenesis is to nucleate cytopharyngeal microtubules which carry dynein-like arms to propel the incoming food. This hypothesis is in line with the suggestions by Eisler (1992) on the significance of inner kinetosomes of the paroral membrane in the nucleation of the postciliary microtubules which in all ciliates (except for the litostomes where transverse microtubules have taken their part) line the cytopharynx.

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